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FOREWORD

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(5) INTRODUCTION

For many patients with mammary cancer the primary tumor can be successfully treated by surgical removal, however the long-term prognosis is not favorable because of the high frequency of metastatic disease which is not treatable by current approaches. We are using tumor-specific immunotherapy to curtail the incidence of metastatic breast cancer. Some of the most efficient anti-tumor mediators are tumor-specific CD8⁺ T lymphocytes. In most cases, for optimal activity CD8⁺ T cells require "help" from antigen-specific CD4⁺ T lymphocytes [1-3]. Recent studies indicate that the inability of the tumor-bearing host to reject tumors may be due to a lack of adequate tumor-specific T_h lymphocytes [3-6]. We have therefore hypothesized that tumor-specific T_h activity can be significantly improved by generating tumor cells that contain all of the necessary antigen presentation, accessory and costimulatory molecules such that they are competent for tumor peptide presentation to CD4⁺ T cells, and thereby facilitate T_h cell activation (reviewed in [7]). Such genetically engineered tumor cells could be used as vaccines to prevent development of metastatic breast cancer, and thereby enhance a host's tumor-specific immune response.

Our strategy is to genetically modify tumor cells so that they can directly present mammary carcinoma tumor peptides to CD4⁺ T helper cells, thereby bypassing the requirement for professional antigen presenting cells and making more efficient the presentation of tumor peptides to T helper lymphocytes (reviewed in [7]). Accordingly, in the *first specific aim* we are using DNA-mediated gene transfer techniques to generate mammary tumor cell transfectants expressing many of the molecules constitutively expressed by professional antigen presenting cells (APC). These molecules include the peptide binding structures or MHC class II molecules, as well as several costimulatory molecules which have been shown to deliver the requisite second signal for T cell activation. The costimulatory molecules to be used include: B7-1 [8, 9], B7-2 [10] and 4-1BB ligand [11-13]. 4-1BB ligand is a very recently described costimulatory molecule that is expressed by professional APC such as macrophages and B lymphocytes. Binding of 4-1BB ligand to its counterreceptor 4-1BB on CD4⁺ and CD8⁺ T cells transmits a potent costimulatory signal to the T cells resulting in T cell activation. Since 4-1BB ligand appears to function independently or synergistically with other costimulatory molecules [11] it appears to be an excellent candidate for coexpression with B7 genes for enhancing tumor-specific immunity. Mammary tumor cells expressing the cytokines IL-1[14] and IL-12 [15, 16] potent inducers of T_{h2} and T_{h1} lymphocytes, respectively, are also being generated. In addition, the gene encoding the bacterial superantigen, SEB, a potent polyclonal T cell activator [17], is being transfected into the mammary tumor lines.

In the *second specific aim* we are determining the tumorigenicity of the transfectants, and their ability to protect the syngeneic host against subsequent challenges of wild type tumor. We will also determine the ability of the transfectants to "rescue" mice carrying established wild type mammary tumors, and identify the helper and effector lymphocytes functional in mammary tumor rejection. In the *third specific aim* we are determining if metastatic mammary cancer can be reduced or prevented by immunization or concomitant treatment with the tumor cell transfectants. This novel tumor-specific immunotherapy approach should significantly improve the host's immune response to autologous breast tumor, and may provide several potential strategies for immune intervention in metastatic mammary cancer.

6. BODY

During years 1-3 of this grant (Aug. 1994 - July 1997) we produced and tested several first generation cell-based vaccines for their ability to limit the growth of established metastatic mouse mammary carcinoma. These first generation vaccines consist of autologous tumor cells transfected with syngeneic MHC class II genes plus costimulatory genes. These vaccines had some immunotherapeutic efficacy in that approximately 60% of mice treated with the vaccines showed significant decreases in metastatic tumor. During the 4th year of the grant (Sept. 1997-August 1998), we have produced and tested a second generation vaccine. This vaccine includes additional genes that facilitate antigen presentation to CD4⁺ T cells (superantigen SEB gene) and is more effective than the first generation vaccine. We have also further refined the mouse model system, the 4T1 mammary carcinoma so that our test system more closely models the clinical setting. In addition, we have tested the IL-1 α transfectants generated during the 3rd year as immunotherapeutic agents. Unfortunately, these cells are not efficacious therapy reagents. Since our second generation vaccines are so effective in our mouse model we are exploring methods for adapting this treatment to the clinic.

The mouse 4T1 mammary carcinoma spontaneously metastasizes to the lungs, liver, brain, lymph nodes, and blood of mice with a primary mammary fat pad tumor. Since mammary tumors in humans frequently metastasize to a variety of sites in the body, we have tested if the 4T1 mouse mammary carcinoma shows a similar metastatic phenotype. Female BALB/c mice were inoculated in the mammary fat pad with varying doses of live 4T1 cells. At weekly intervals beginning 2 weeks after tumor challenge, mice were sacrificed and the lungs, liver, blood, brain, and draining lymph nodes removed and tested by the clonogenic assay for metastatic cells. As shown in Figure 1, by 2 weeks after initial

inoculation 4T1 tumor cells have metastasized to the draining lymph nodes and lungs, and by week 4 they have metastasized to the blood, liver, and brain. We conclude from these data that 4T1 metastasizes very early after primary tumor inoculation and that the pattern of metastasis is very similar to that of human breast cancers. These results further support the validity of using the 4T1 mouse mammary tumor as an animal model for human disease.

We have produced 2nd generation cell-based vaccines consisting of autologous tumor cells transfected with syngeneic MHC class II, CD80, and SEB genes.

Superantigens, such as SEB, are polyclonal activators of T lymphocytes and have been shown to activate tumor-specific T cells [17]. We have, therefore, reasoned that 4T1 tumor cells expressing the SEB gene should be effective cell-based vaccines for activating tumor-specific T lymphocytes. The gene

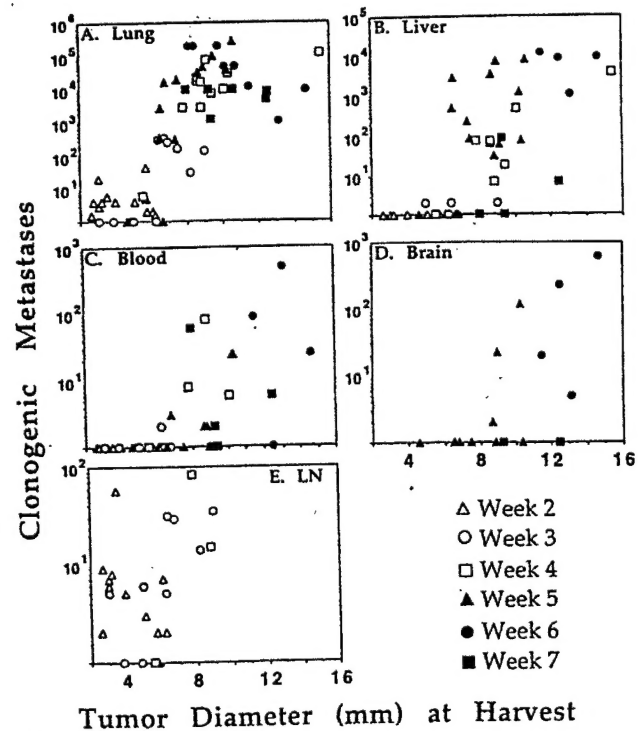


Figure 1: 4T1 tumor cells spontaneously metastasize to the lungs (panel A), liver (panel B), blood (panel C), brain (panel D), and LN (panel E). BALB/c mice were inoculated in the mammary fat pad with 5×10^3 wild type 4T1 cells and sacrificed at 2-7 weeks after initial tumor challenge. The number of metastatic cells was determined using the clonogenic assay.

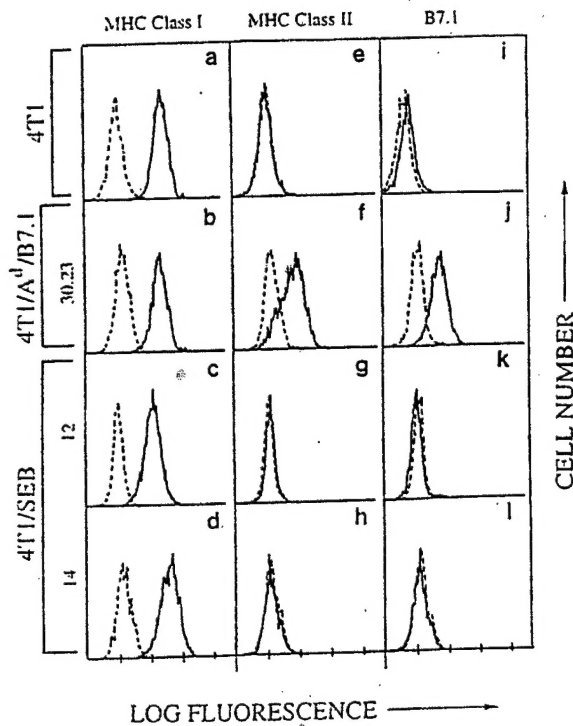


Figure 2. Flow cytometry analysis of 4T1 transfectants stained for MHC class I, MHC class II, and B7.1 expression using indirect immunofluorescence.

BALB/c mice in the mammary fat pad with wild type 4T1 tumors, and approximately 3 weeks later began therapy with the SEB transfectants. Figure 3 shows the results of these experiments and demonstrates that the greatest reduction in number of lung metastases occurs when mice are treated with

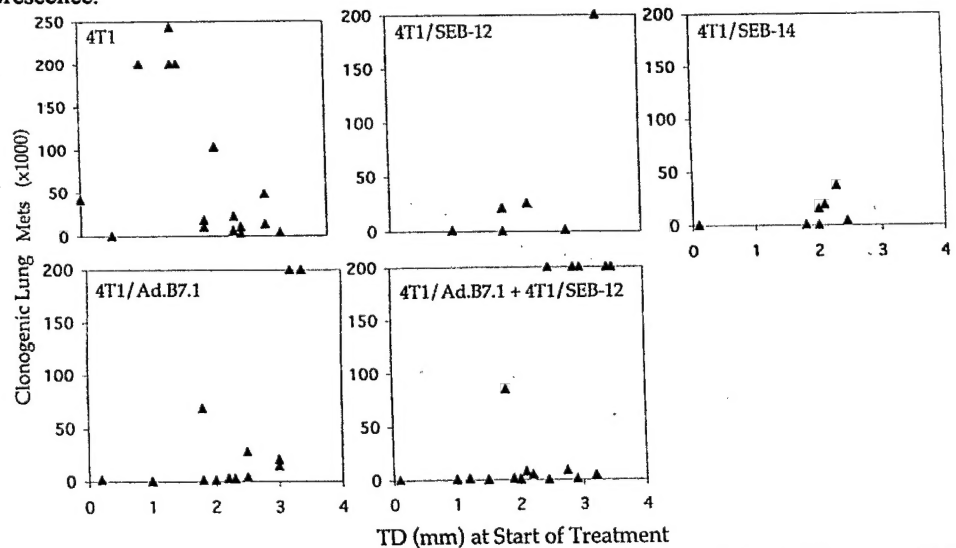


Figure 3. BALB/c mice with established spontaneous wild type 4T1 metastases treated with 4T1/A^d/B7.1 + 4T1/SEB transfectants have reduced number of lung metastases. Mice were inoculated in the mammary fat pad with wild type 4T1 and therapy with transfectants was started approximately 3 weeks later when primary tumors reached the indicated sizes.

cloning and SEB secretion of these cells were described in last year's progress report. We have now performed immunofluorescence analysis to characterize these cells with respect to MHC class I, class II, and CD80 expression. As shown in Figure 2, the 4T1/A^d/SEB transfectants express MHC class II molecules and the 4T1/A^d/B7.1/SEB transfectants express MHC class II + B7.1 (CD80) molecules. All 4T1 cells express MHC class I molecules.

The second generation vaccines are effective immunotherapeutic agents for the treatment of mice with established metastatic disease and primary tumors. To compare the second generation vaccines to our initial vaccines, we have inoculated

4T1/A^d/B7.1 + 4T1/SEB transfectants. It should be noted that at the time of initiation of immunotherapy, the mice already have large deposits of established metastatic tumor in the lungs.

Following surgical removal of a primary 4T1 breast carcinoma, BALB/c mice develop metastatic disease. Lethality as a result of breast cancer in women is due to metastatic disease, not primary tumor, since primary tumor can be surgically removed. We have, therefore, developed a mouse model system to parallel human disease and to test our cell-based vaccines in a more clinically relevant setting. BALB/c mice are inoculated in the mammary fat pad with 7×10^3 4T1 tumor cells. The primary tumors are allowed to grow progressively and are removed surgically when they reach approximately 2-8 mm in diameter. The post-surgery mice are then followed for recurrence of primary tumor and for the development of metastatic disease. Approximately 80-85% of mice undergoing surgery survive the procedure and live until they die from metastatic disease; primary tumor very rarely occurs at the site of surgery (<2% of operated mice). Figure 4 shows the survival of post-surgery mice as a function of primary tumor size at the time of surgery. Mice which do not have their tumors surgically removed die at approximately day 47 after initial implantation of tumor. Note that regardless of tumor size at surgery, the post-surgery mice all die at approximately 47 days after initial inoculation of primary tumor in the mammary fat pad. This observation indicates that the 4T1 tumor metastasizes very early after implantation, and that mice die from the cells that metastasize very early on. These results agree well with our previous studies which have demonstrated that 4T1 cells rapidly metastasize to the draining lymph node following inoculation of primary tumor in the mammary fat pad. In our experimental system, establishment of distant metastases following surgical removal of primary tumor is, therefore, comparable to the clinical situation where women develop metastatic disease after removal of their primary tumor. Since this animal model appears to closely mimic human metastatic disease, we will use it to test our cell-based vaccines.

Therapy of post-surgery mice with the second generation 4T1/A^d/B7.1 + 4T1/SEB

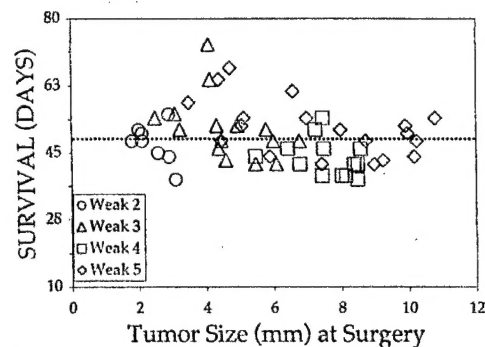


Figure 4. Mean survival times of BALB/c mice following removal of primary tumor. Mice were inoculated in the mammary fat pad with 7×10^3 wild type 4T1 cells. Primary tumors were surgically removed at the indicated sizes, and mice were followed for survival relative to control treated mice.

transfectants significantly extends mean survival time. We have tested the immunotherapeutic potency of the SEB transfectants in syngeneic BALB/c mice following surgical removal of their primary tumor. Mice were initially inoculated with 7×10^3 wild type 4T1 cells in the mammary fat pad and the tumors allowed to grow for approximately 3 weeks until they ranged in size from 2-6 mm in diameter. Primary tumors were then surgically removed and beginning one week after surgery (4 weeks after initial 4T1 inoculation) therapy was initiated. Mice were then treated once a week for 3 weeks with irradiated transfectants. As shown in Figure 5 the immunotherapy regimen with 4T1/A^d/B7.1 + 4T1/SEB transfectants significantly extends mean survival time as compared to mice treated with wild type 4T1 cells. Previous experiments (data not shown) demonstrate that the SEB/class II/B7.1 transfectants are more potent than transfectants containing individual genes.

Since our cell-based vaccines give promising results in the mouse model system, we would like to extend these studies to human mammary cancer patients. As a prelude to extending our vaccination approach to women with metastatic breast cancer, we are screening human mammary cancer cell lines for an appropriate line to use as the "base" line for the vaccine. Since it is not feasible (too time consuming and too costly) to use autologous tumor cells for the vaccine, we will identify candidate established human breast cell lines that could be genetically modified as vaccines. Based on our previous studies [18-20] the optimal vaccine will consist of tumor cells that constitutively express MHC class II, but do not co-express the invariant chain (Ii) gene. The vaccine must be MHC class II semi-syngeneic with respect to the patient being treated so that class II-restricted CD4⁺ T cells are activated. The vaccine must also express a tumor antigen shared with the patient's tumor antigen. To satisfy these conditions we are screening established human breast tumor lines for cells that express Her2/neu and do not constitutively express MHC class II genes and are not inducible for expression by interferon- γ since IFN- γ also induces Ii chain. Candidate cell lines will

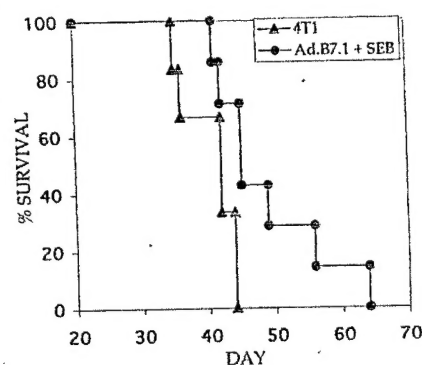


Figure 5. Therapy with 4T1/SEB + 4T1/A^d/B7.1 transfectants significantly increases mean survival time of mice whose primary mammary tumors were surgically removed. Mice were inoculated in the mammary fat pad with 7×10^3 wild type 4T1 tumor cells and tumors allowed to grow for 3 weeks. Primary tumors were then surgically removed and immunotherapy with transfectants was started one week later.

be transfected with a variety of common human HLA-DR genes, the CD80 gene, and the SEB gene. Patients will be candidates for receiving this vaccine if they have her2/neu⁺ tumors and share an HLA-DR allele with the vaccine.

In our preliminary studies we have screened several human breast tumor lines. One line, SUM185PT, kindly supplied by Dr. Steven Ethier at the U. of Michigan via a DOD breast cancer grant, meets our criteria. As shown in figure 6, SUM185PT cells do not express MHC class II or Ii molecules either constitutively or following exposure to 100 or 1000 units/ml IFN- γ . As shown in figure 7, the SUM185PT cells also express her2/neu antigen as measured by flow cytometry using an anti-Her2/neu mAb.

Since the SUM185PT cells will be transfected with a variety of genes to generate the cell-based vaccine, we have also tested their transfectability using a variety of lipid fusing agents marketed by BRL as the "Perfect Transfection Kit." As shown in figure 8, several of the lipid-fusing agents mediate transfection and transient expression of a plasmid containing the green fluorescence protein (GFP). We are optimistic that we will be able to generate stable transfectants using one of these lipid fusing agents.

Although studies from year 3 indicated that 4T1 tumor cells expressing the IL-1 α gene have reduced tumorigenicity, these cells have not proven effective as cell-based vaccines for the treatment of established metastatic disease. We tested

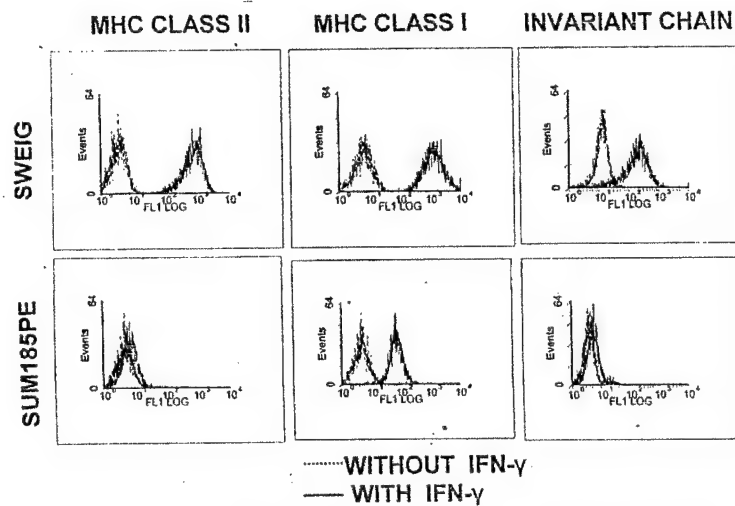


Figure 6. The human breast cancer cell line SUM185PT does not constitutively express MHC class II or invariant (Ii) chain molecules and is not inducible for expression by IFN-gamma. Cells were stained by indirect immunofluorescence and antigen expression monitored by flow cytometry.

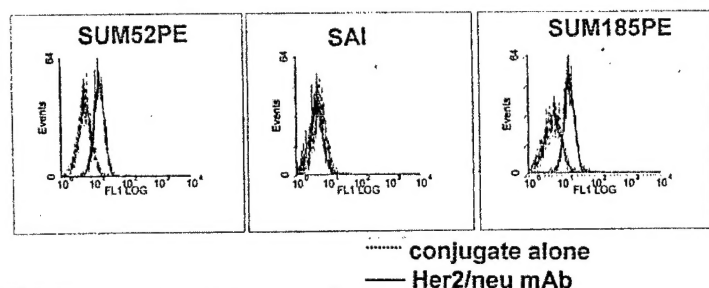


Figure 7. Human breast cancer cell line SUM185PT expresses Her2/neu. Cells were stained for Her2/neu expression using the c-erb2 mAb.

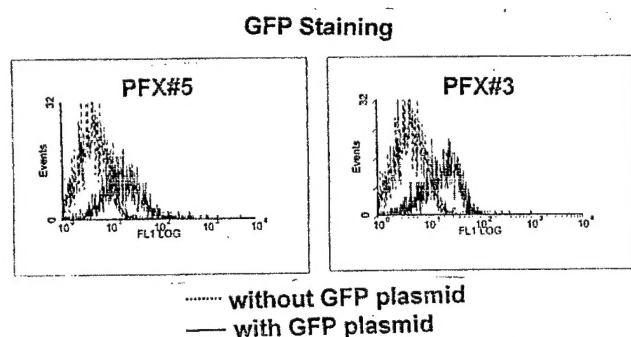


Figure 8: SUM185PT human mammary cancer cells are highly transfectable. Cells were transfected with a variety of lipid fusing agents and a plasmid containing the GFP gene. Cells were analyzed for GFP expression 2 days after transfection by flow cytometry.

adapted it to more closely model human metastatic breast cancer. We have used

the therapeutic efficacy of 4T1/IL-1 α transfectants as follows: Syngeneic BALB/c mice were inoculated in the mammary fat pad with wild type 4T1 tumor cells and the tumors allowed to grow to approximately 3-5 mm in diameter at which time lung metastases were already present. Tumor-bearing mice were then treated once a week for 3 weeks with irradiated transfectants and the number of metastatic cells in the lungs and liver determined by the clonogenic assay after 3 weeks of treatment. As shown in figure 9, there is no difference between therapy with 4T1/IL-1 α vs. therapy with wild type 4T1 cells, indicating that the IL-1 α transfectants have no significant therapeutic benefit. Similar results were obtained when the IL-1 α transfectants were combined with 4T1/A^d transfectants (data not shown).

7. CONCLUSIONS:

During this 4th grant year we have further refined the mouse mammary carcinoma 4T1 tumor system and

this animal model to test our second generation vaccines, some of which show potent anti-metastatic effects in vivo in mice with spontaneous, established metastatic breast cancer.

Because we see significant reductions, and in some cases elimination, of metastatic disease in treated mice, we view these newly developed cell-based vaccines as very promising immunotherapeutic agents. We have begun to design strategies for extending this therapeutic approach to the clinic by screening human breast cancer cell lines for suitable "base" lines for our cell-based vaccines.

Earlier this year we requested a no-cost extension for a 5th year for this project. This request was approved so we will be continuing these studies through August 1999. We have several goals for the final year of this grant. 1) We will continue the therapy studies and try to develop 3rd generation vaccines and more effective protocols for reducing metastatic disease in the mouse 4T1 model system. 2) Our initial application included another set of transfectants expressing the 4-1BBL costimulatory molecule. Because we have been so busy with the SEB, class II, and CD80

transfectants we have not, as yet, tested the 4-1BBL transfectants. We hope to test these novel transfectants during the coming year. 3) Since some of the cell-based vaccines have potent immunotherapeutic activity, we will identify the immune cells that mediate metastasis regression in these therapy settings. These studies will expand to include assays of NK cells NK1.1 V α 14⁺ T cells that recognize antigen in the context of CD1 molecules, and standard antibody depletion

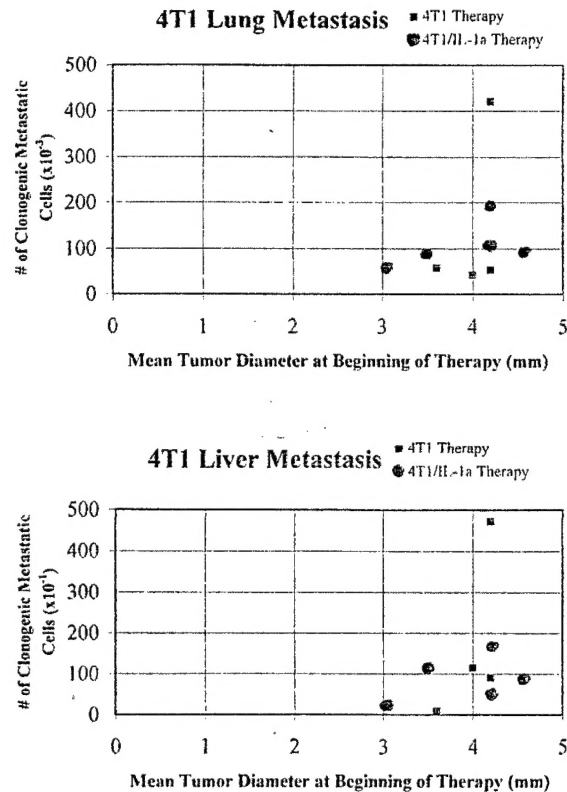


Figure 9: Immunotherapy with 4T1/IL-1 α transfectants does not significantly reduce metastatic disease. BALB/c mice were inoculated in the mammary fat pad with 7×10^3 wild type 4T1 cells and therapy with transfectants and control cells started when tumors were 2-6 mm in diameter. Therapy consisted of 3 weekly injections of irradiated cells. Number of metastatic cells in the liver and lung was determined using the clonogenic assay.

studies for conventional CD4⁺ and CD8⁺ T lymphocytes. We feel that all of these cell populations are potentially involved in metastasis regression because preliminary studies indicate an increase in NK1.1⁺ and NK1.1⁺CD3⁺ cells and an inability of CD4⁺ and CD8⁺ T cell depletion to fully reduce the therapeutic effect. 4) We will continue to screen human mammary cancer cell lines for candidate "base" lines for translating this immunotherapy approach to the clinic. We have submitted a new DOD Army Breast Cancer application to fund these basic research/translational studies. These studies will include generation of the human cell-based vaccines and their in vitro testing with human PBL's from mammary cancer patients to determine the vaccines' ability to stimulate anti-tumor immunity. We anticipate that these studies will provide the necessary background information for a clinical trial of these novel vaccines in breast cancer patients.

(8) References

1. Kern, D., J. Klarnet, M. Jensen, and P. Greenberg. 1986. Requirement for recognition of class II molecules and processed tumor antigen for optimal generation of syngeneic tumor-specific class I-restricted CTL. *J. Immunol.* 136:4303-.
2. Keene, J. and J. Forman. 1982. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J. Exp. Med.* 155:768-782.
3. Schultz, K., J. Klarnet, R. Gieni, K. Hayglass, and P. Greenberg. 1990. The role of B cells for in vivo T cell responses to a friend virus-induced leukemia. *Science* 249:921-.
4. Ostrand-Rosenberg, S., A. Thakur, and V. Clements. 1990. Rejection of mouse sarcoma cells after transfection of MHC class II genes. *J. Immunol.* 144:4068-4071.
5. Tepper, R., P. Pattengale, and P. Leder. 1989. Murine IL-4 displays potent anti-tumor activity in vivo. *Cell* 57:503.
6. Fearon, E., D. Pardoll, T. Itaya, P. Golumbek, H. Levitsky, J. Simons, H. Karasuyama, B. Vogelstein, and P. Frost. 1990. IL-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* 60:397-403.
7. Ostrand-Rosenberg, S. 1994. Tumor immunotherapy: The tumor cell as an antigen-presenting cell. *Curr. Opin. Immunol.* 6:722-727.
8. Thompson, C.B. 1995. Distinct roles for the costimulatory ligands B7-1 and B7-2 in T helper cell differentiation? *Cell* 81:979-982.
9. June, C.H., J.A. Bluestone, L.M. Nadler, and C.B. Thompson. 1994. The B7

- and CD28 receptor families. *Immunology Today* 15:321-331.
10. Razi-Wolf, Z., F. Galvin, G. Gray, and H. Reiser. 1993. Evidence for an additional ligand, distinct from B7, for the CTLA-4 receptor. *Proc. Natl. Acad. Sci. USA* 90:11182-11186.
 11. De Benedette, M., N. Chu, K. Pollock, J. Hurtado, W. Wade, B. Kwon, and T. Watts. 1995. Role of 4-1BB ligand in costimulation of T lymphocyte growth and its upregulation of M12 B lymphomas by cAMP. *J. Exp. Med.* 181:985-992.
 12. Pollock, K., Y. Kim, Z. Zhou, J. Hurtado, K. Kim, R. Pickard, and B. Kwon. 1993. Inducible T cell antigen 4-1BB. *J. Immunol.* 150:771.
 13. Pollock, K., Y. Kim, J. Hurtado, Z. Shou, K. Kim, and B. Kwon. 1994. 4-1BB T cell antigen binds to mature B cells and macrophages and costimulates anti-mu primed splenic B cells. *Eur. J. Immunol.* 24:367.
 14. Gruber, M., D. Webb, and T. Gerrard. 1992. Stimulation of human monocytes via CD45, CD44, and LFA-3 triggers MCSF production: synergism with lipopolysaccharides and IL-1beta. *J. Immunol.* 148:1113.
 15. Manetti, R., P. Parronchi, M. Guidizi, M. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural Killer cell stimulatory factor (IL-12) induces T helper type 1 specific immune response and inhibits the development of IL-4 producing T helper cells. *J. Exp. Med.* 177:1199.
 16. Schoenhaut, D., A. Chua, A. Wolitzky, P. Quinn, C. Dwyer, W. McComas, P. Familletti, M. Gately, and U. Gubler. 1992. Cloning and expression of murine IL-12. *J. Immunol.* 148:3813.
 17. Shu, S., R. Krinock, T. Matsumura, J. Sussman, B. Fox, A. Chang, and D. Terman. 1994. Stimulation of tumor-draining lymph node cells with superantigenic staphylococcal toxins leads to the generation of tumor-specific effector T cells. *J. Immunol.* 152:1277-1288.
 18. Armstrong, T., V. Clements, B. Martin, J.P.-Y. Ting, and S. Ostrand-Rosenberg. 1997. Major histocompatibility complex class II-transfected tumor cells present endogenous antigen and are potent inducers of tumor-specific immunity. *Proc. Natl. Acad. Sci. USA* 120:123-128.
 19. Armstrong, T., V. Clements, and S. Ostrand-Rosenberg. 1998. MHC class II-transfected tumor cells directly present antigen to tumor-specific CD4⁺ T lymphocytes. *J. Immunol.* 160:661-666.
 20. Armstrong, T., B. Pulaski, and S. Ostrand-Rosenberg. 1998. Tumor antigen presentation: Changing the rules. *Canc. Immunol. Immunother.* 46:70-74.